



fit4nano

Newsletter # 4 - April 2024

© Priyana Puliappara Babu, Universität Konstanz

CONTENT

Editorial

Newsletter number 4 is out early to announce our upcoming activities and events (and more importantly, upcoming deadlines!) rather than reporting on the past.

Applications for the FIT4NANO FIB summer school can still be submitted until next Wednesday, 1st of May. Please spread the word to PhD students you think might be interested.

There is a bit more time to sign up for our two working group meetings in June (WG2 & WG3). Participation is possible on-site or online. Last, but not least, the deadline for abstract submission for this year's workshop in Albania is approaching fast. You have one more month to submit your contribution ,the deadline being on the 31st of May.

We hope to see many, if not all of you, at one event or another!

Gemma Rius, Institute of Microelectronics of Barcelona (IMB-CNM-CSIC)



PROJECT NEWS

- WG MEETING ANNOUNCEMENTS
- TRAINING SCHOOL
- WORKSHOP'24



Member news

- PUBLICATION
Gas Phase Synthesis of Iron Silicide Nanostructures using a Single-Source Precursor: Comparing Direct-Write Processing and Thermal Conversion

- ITC Conference Grant Report by Sara Metwally

www.fit4nano.eu



fit4nano project news

Working Group Meeting Announcements

WG1

The working group 1 meeting will take place at ISI in Brno in the beginning of September and will focus on topics such as CPO development (Lecture, simulation, optimization, design), Ion source and FIB, Electron source and SEM/TEM, Spectrometer (energy and mass). More information will be released shortly on the FIT4NANO website.

WG2

A work group 2 meeting is scheduled in Vienna for June 28, 2024, with the theme **Towards realistic secondary electron simulation**. The goal of the meeting is to discuss paths towards refined simulation of FIB induced secondary electron generation, emission, and FIBID. The event will feature invited talks on existing solutions and possible contributions to model enhancement from experiments and more fundamental theories. A discussions session will conclude the day. Further information and information on registration can be found at <https://events.hifis.net/event/1475>

WG3

The last meeting of the Applications Working Group (WG3) takes place in Berlin on June 13-14. The topic of the WG3 meeting is **Software development, pattern generation and automation for FIB applications**. Therewith, we will not only address important current developments for FIB, but also the framework for a possible future COST Action application on 3D printing will be discussed.

Further information can be found here: <https://fit4nano.eu/fit4nano-events/wg3-meeting-2/>

If you would like to attend, please register here: <https://events.hifis.net/event/1451/>

WG4

A series of online interviews with authors of the **Roadmap for focused ion beam technologies** is set to take place this summer. A roundtable discussion about the roadmap is planned for the FIT4NANO workshop in Albania.

Due to budget restraints, reimbursement of travel costs is possible for invited speakers only.

2nd Role Model Webinar Report

In February 2024, we hosted our 2nd Role Model Webinar featuring 4 speakers working in industry, science management, and academia:

Prof. Assoc. Bergin Gjonaj, Albanian University

Prof Karen Kavanagh, Simon Fraser University, CA

Dr Mafalda Quintas, Science Officer at COST

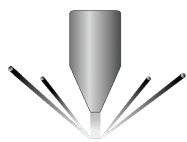
Dr Adam V. Steele, zeroK NanoTech

[A recording of the video can be viewed here](#)

STSM Call

Applications for STSMs are still accepted until the available budget is used up. We can fund ~2 more STSMs this GP. Please be aware that STSMs have to be finished by the beginning of October as the GP ends in mid-October.

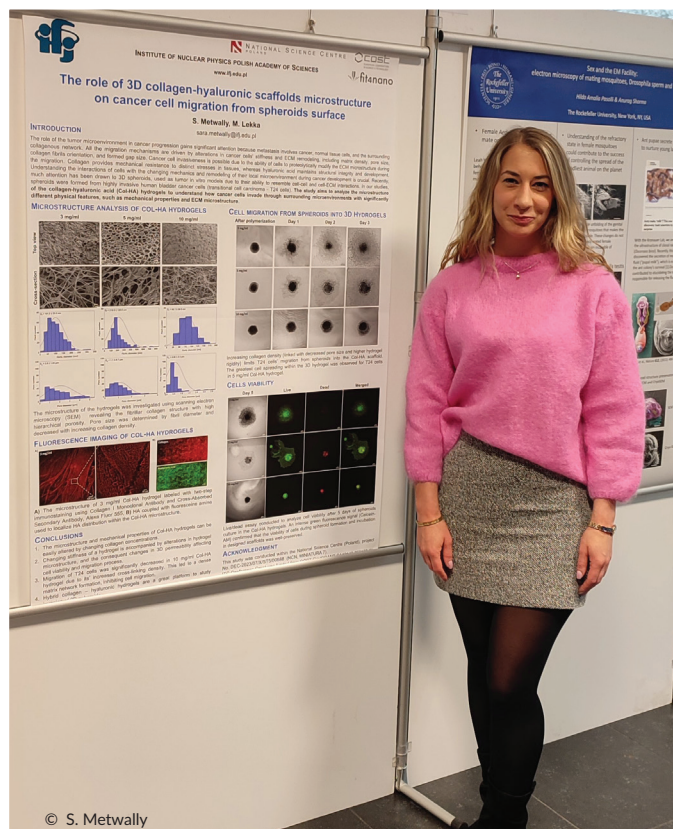
Applications will be reviewed and assessed in the order they are submitted. All information and rules concerning STSMs can be found at <https://fit4nano.eu/stsm/>



ITC Conference Grant Report by Sara Metwally

Recently, I had the pleasure of participating in the conference “From 3D Light to 3D Electron Microscopy”, thanks to ITC Conference Grants. This interdisciplinary meeting covered correlative light and electron microscopy with a significant focus on advanced techniques for 3D volume imaging of biological samples, including Focused Ion Beam (FIB) and cryo-FIB techniques.

I had an opportunity to present my recent study on microstructure analysis of a complex biological system consisting of 3D collagen-hyaluronic hydrogels mimicking the natural extracellular matrix (ECM) with embedded cancer cell spheroids, designed to study the role of ECM in cancer cell migration and invasion model (National Science Centre projects: MINIATURA 7 entitled “Microstructural analysis of collagen-hyaluronic scaffolds for studying ECM reorganization in cancer invasion model,” No. DEC-2023/07/X/ST5/00688, and Opus 21 entitled “Collagen-hyaluronic gels as an environment for a controlled change of mechanical properties of spheroids under shear forces” No. UMO-2021/41/B/ST5/03032).



During two Poster Sessions, I had a great discussion with experts in correlative light and electron microscopy and received valuable feedback from both scientific and industry experts. This conference was a great opportunity to expand my knowledge and learn more about sample preparation techniques, staining procedures, and volume imaging of biological samples, such as individual organelles, cells, and tissues, using FIB and cryo-FIB techniques. This event combined scientific sessions with a broad range of workshops where correlative workflows, tools, tips, and tricks were shared between participants and trainers/speakers. During the workshops, I learned about other correlative and multimodal volume imaging techniques, enabling more detailed insights into biological processes (e.g., multi-beam SEM imaging, SEM-Array tomography, and Femtosecond laser milling for nanoscale imaging). This was also an excellent opportunity to learn about data analysis, segmentation, and visualization of volume FIB-SEM datasets, including deep machine learning and artificial intelligence (AI) tools that recently became powerful tools in studies of nanostructured functional materials. Participating in this conference was a great experience. The knowledge I gained will allow me to apply new techniques to my studies and improve my future research. It also helped me establish new contacts for future collaborations and broaden the awareness of FIT4NANO Cost Action within the life science community.



NATIONAL SCIENCE CENTRE
INSTITUTE OF NUCLEAR PHYSICS POLISH ACADEMY OF SCIENCES
www.ifj.edu.pl



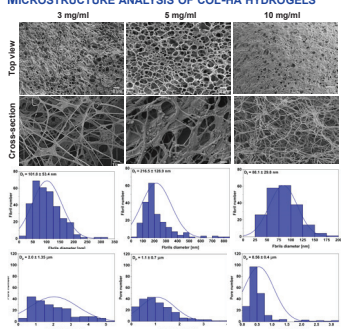
The role of 3D collagen-hyaluronic scaffolds microstructure on cancer cell migration from spheroids surface

S. Metwally, M. Lekka
sara.metwally@ifj.edu.pl

INTRODUCTION

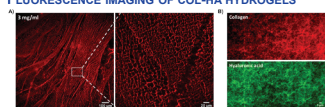
The role of the tumor microenvironment in cancer progression gains significant attention because metastasis involves cancer, normal tissue cells, and the surrounding collagenous network. All the migration mechanisms are driven by alterations in cancer cells' stiffness and ECM remodeling, including matrix density, pore size, collagen fibrils orientation, and formed gap size. Cancer cell invasiveness is possible due to the ability of cells to proteolytically modify the ECM microstructure during the migration. Collagen provides mechanical resistance to distinct stresses in tissues, whereas hyaluronic acid maintains structural integrity and development. Understanding the interactions of cells with the changing mechanics and remodeling of their local microenvironment during cancer development is crucial. Recently, much attention has been drawn to 3D spheroids, used as tumor in vitro models due to their ability to resemble cell-cell and cell-ECM interactions. In our studies, spheroids were formed from highly invasive human bladder cancer cells (transitional cell carcinoma - T24 cells). The study aims to analyze the microstructure of the collagen-hyaluronic acid (Col-HA) hydrogels to understand how cancer cells invade through surrounding microenvironments with significantly different physical features, such as mechanical properties and ECM microstructure.

MICROSTRUCTURE ANALYSIS OF COL-HA HYDROGELS



The microstructure of the hydrogels was investigated using scanning electron microscopy (SEM) revealing the fibrillar collagen structure with high hierarchical porosity. Pore size was determined by fibril diameter and decreased with increasing collagen density.

FLUORESCENCE IMAGING OF COL-HA HYDROGELS

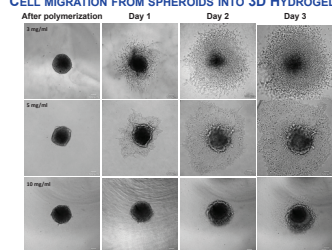


A) The microstructure of 3 mg/ml Col-HA hydrogel labeled with two-step immunostaining using Collagen I Monoclonal Antibody and Cross-Absorbed Secondary Antibody, Alexa Fluor 555. B) HA coupled with fluorescently amine used to localize HA distribution within the Col-HA microstructure.

CONCLUSIONS

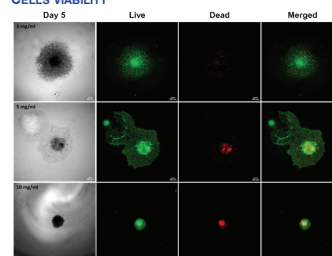
- The microstructure and mechanical properties of Col-HA hydrogels can be easily altered by changing collagen concentrations.
- Changing stiffness of a hydrogel is accompanied by alterations in hydrogel microstructure, and the consequent changes in 3D permeability affecting cell viability and migration process.
- Migration of T24 cells was significantly decreased in 10 mg/ml Col-HA hydrogel due to its' increased cross-linking density. This led to a dense matrix network formation, inhibiting cell migration.
- Hybrid collagen - hyaluronic hydrogels are a great platform to study invasion of 3D spheroids.

CELL MIGRATION FROM SPHEROIDS INTO 3D HYDROGELS



Increasing collagen density (linked with decreased pore size and higher hydrogel rigidity) limits T24 cells' migration from spheroids into the Col-HA scaffold. The greatest cell spreading within the 3D hydrogel was observed for T24 cells in 5 mg/ml Col-HA hydrogel.

CELLS VIABILITY



Live/dead assay conducted to analyze cell viability after 5 days of spheroids culture in the Col-HA hydrogels. An intense green fluorescence signal (Calcein-AM) confirmed that the viability of cells during spheroid formation and incubation in designed scaffolds was well-preserved.

ACKNOWLEDGMENT

This study was conducted within the National Science Centre (Poland), project No. DEC-2023/07/X/ST5/00688 (N.N., MINIATURA 7). ITC Conference Grant was funded from CoST-UE (GRANT-CA19140-25818043)

Gas Phase Synthesis of Iron Silicide Nanostructures using a Single-Source Precursor: Comparing Direct-Write Processing and Thermal Conversion

Felix Jungwirth^{1,2}, Alba Salvador-Porroche³, Fabrizio Porrati¹, Nicolas P. Jochmann^{1,2}, Daniel Knez⁴, Michael Huth¹, Isabel Gracia⁵, Carles Cané⁵, Pilar Cea^{3,6}, José María De Teresa³, Sven Barth^{1,2}

1 Institute of Physics, Goethe University Frankfurt, Max-von-Laue-Str. 1, 60323 Frankfurt am Main, Germany.

2 Institute for Inorganic and Analytical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany.

3 Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, 50009 Zaragoza, Spain.

4 Institute of Electron Microscopy and Nanoanalysis, Graz University of Technology, Steyrergasse 17, 8010 Graz, Austria.

5 Institut de Microelectrònica de Barcelona (IMB), Centre Nacional de Microelectrònica (CNM), Consejo Superior de Investigaciones Científicas (CSIC), 08193 Barcelona, Spain.

6 Laboratorio de Microscopías Avanzadas (LMA), Universidad de Zaragoza, Edificio de I+D+i, Campus Río Ebro, 50018 Zaragoza, Spain.

The investigation of precursor classes for the fabrication of nanostructures are of specific interest for maskless fabrication and direct nanoprinting by focused ion and electron beam induced deposition (FIBID/FEBID). This article reports on specific differences in the composition and microstructure of the deposits, when the $(\text{H}_3\text{Si})_2\text{Fe}(\text{CO})_4$ precursor is converted to an inorganic material by electron, ion and thermal induced processes. Maximum metal/metalloid contents of up to 90 at% are obtained in FIBID deposits and higher than 90 at% in CVD films, whereas FEBID with the same precursor provides material containing less than 45 at% total metal/metalloid content (Fig.1). Moreover, the Fe:Si ratio is maintained well in both FEBID and CVD processes, but FIBID using Ga⁺ ions liberates more than 50% of the initial Si provided by the precursor. This, in combined with the previous result in the Co-Si system, suggests that precursors for FIBID processes targeting binary materials should include multiple bonding to maintain the predefined metal/metalloid ratio, such as bridging positions for lighter non-metals as previously described $(\text{Co}(\text{CO})_4)_2\text{SiH}_2(\text{Co}(\text{CO})_4)_2$.

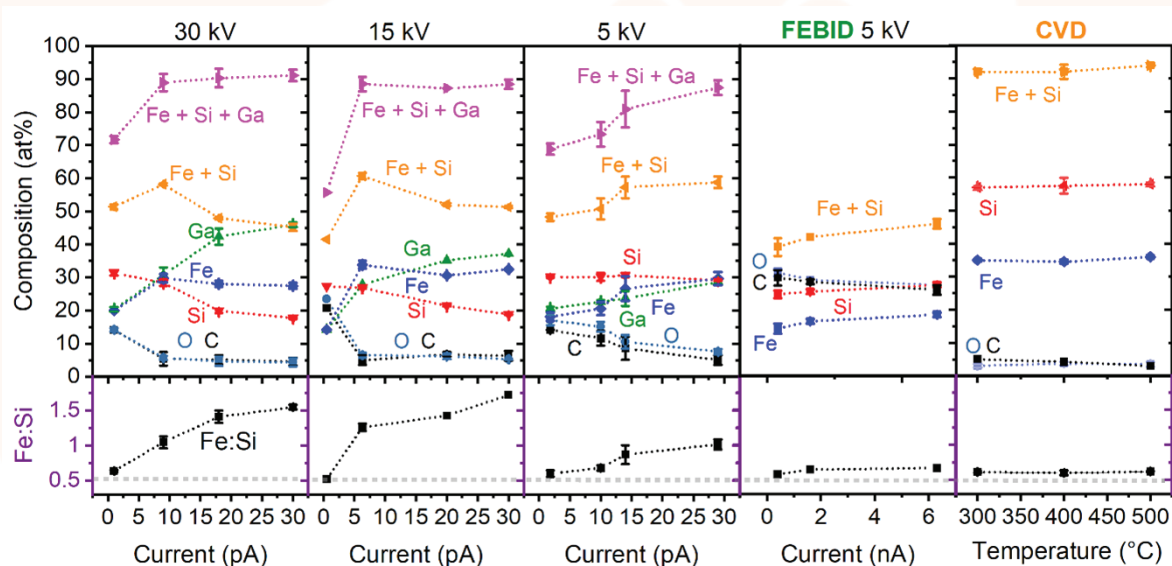


Fig.1 Comparison of EDX data obtained for deposits prepared by different techniques, while also varying further beam parameters. FIBID, FEBID and CVD were performed using $(\text{H}_3\text{Si})_2\text{Fe}(\text{CO})_4$ as single source precursor.

F. Jungwirth, A. Salvador-Porroche, F. Porrati, N. P. Jochmann, D. Knez, M. Huth, I. Gracia, C. Cané, P. Cea, J. M. De Teresa, S. Barth. *J. Phys. Chem. C* 2024, 128, 7, 2967–2977.

DOI: <https://doi.org/10.1021/acs.jpcc.3c08250>



FIT4NANO Summer School 2024

FIT4NANO is hosting its 2nd summer school from 11-16 August in Thun, Switzerland. Practical training and lab visits complement the planned lectures on various topics like FIBID application and simulation, FIB instrumentation, Charged Particle Optics, Introduction to Simulation methods, SIMS, TOFSIMS, and Post-processing of data.

The school is organized by Ivo Utke (Empa | Swiss Federal Laboratories for Materials Science and Technology) and Silvia Schintke (HEIG-VD / HES-SO, University of Applied Sciences Western Switzerland).

Confirmed lecturers:

Katja Höflich, Ferdinand-Braun-Institut
 Gregor Hlawacek, HZDR
 Florent Houdellier, CEMES-CNRS
 Silvan Kretschmer, HZDR
 Lex Pillatsch, Tofwerk
 Valentine Riedo-Grimaudo, Tofwerk
 Ivo Utke, EMPA
 James Whitby, Tofwerk



Participants of the 2022 summer school during hands-on training © FIT4NANO

Successful applicants will receive a travel grant to cover their expenses. Online participation will be possible to attend the lectures.

Applications for both in-person and online participation can be submitted until 1st of May, 12 p.m. (noon)

Further information is available at <https://fit4nano.eu/summer-school-2024/>

4th FIT4NANO Workshop

The 2024 workshop will take place from 16 - 20 September at the Adriatic Hotel in Durres, Albania. The workshop is hosted by Genta Rexha, Barleti University. As in GP1, the workshop will be co-organized with the European FIB Network Eu-F-N (<https://www.eu-f-n.org>). Expect four to five days of presentations, poster sessions and FIT4NANO Working Group meetings.

Confirmed invited speakers:

- » Roland Bruetsch, Paul-Scherrer-Institut, Switzerland - HotFIB
- » Santhana Eswara, LIST, Luxembourg - in operando battery characterization – opincharge
- » Ewelina Gacka, University of Wroclaw, Poland - FIBID MEMS sensors
- » Vivek Goyal, Boston University, MA, USA - Shot noise-mitigated secondary electron imaging with ion count-aided microscopy
- » Michael Grange, Rosalind Franklin Institute, UK - Cryo-plasma FIB for biological samples
- » Brenton Knuffman, zeroK Nanotech, USA - FIB-SEM-SIMS imaging in a slice and view approach
- » Alexander Rigort, Thermofisher - Developments in the field of Cryo-Plasma-FIB

Further information is available at <https://fit4nano.eu/fit4nano-eufn-workshop-2024/>

Abstract submission and registration is open at <https://events.hifis.net/event/1162/>

Abstracts can be submitted until 31 May, registration for on-site participation closes on 19 August.

If you have questions, comments, or input for the next newsletter, please send an e-mail to Astrid at a.berens@hzdr.de

Newsletter design by Astrid Berens. Title page design adapted from www.stockindesign.com